Fuel xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Fuel

journal homepage: www.elsevier.com/locate/fuel

# Ethanol fermentation characteristics of *Pichia stipitis* yeast from sugar beet pulp hydrolysate: Use of new detoxification methods

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## HIGHLIGHTS

- Sugar beet pulp is a promising feedstock for second generation ethanol production.
- New detoxification methods were tested for inhibitor removal from the hydrolysate.
- Effect of detoxification method on ethanol production was discussed.
- Kinetic constants were found for both synthetic and hydrolysate growth media.
- Ethanol was produced with yields on sugar beet pulp between 0.108 and 0.122 g $g^{-1}$.

## ARTICLE INFO

*Article history:*
Received 25 January 2015
Received in revised form 3 June 2015
Accepted 4 June 2015
Available online xxxx

*Keywords:*
Bioethanol
Hemicellulose
Hydrolysate
Fermentation
*Pichia stipitis*

## ABSTRACT

This study investigates the ethanol production characteristics of adapted *Pichia stipitis* yeast in growth media containing various sugar sources, namely, synthetic xylose, sulfuric acid hydrolyzed sugar beet pulp and sugar beet pulp hydrolysate–synthetic xylose mixture. In synthetic growth media, the xylose concentration range of 10–75 g $l^{-1}$ did not exhibit substrate inhibition and at an initial xylose concentration of 75.1 g $l^{-1}$, 37.1 g $l^{-1}$ ethanol was obtained after 75 h with productivity of 0.494 g $l^{-1}$ $h^{-1}$. Prior to fermentation, the inhibitory components of the sugar beet pulp hydrolysate was detoxified using three different treatments: commercial activated charcoal with overliming, activated charcoal obtained from sugar beet pulp with overliming and fly ash treatment. Phenolics and furan compounds were significantly adsorbed with activated charcoal treatments and overliming with CaO showed to enhance their reduction. A significant amount of furan was removed by fly ash treatment due to its high CaO content. Neither case demonstrated a noticeable decrease in acetic acid concentration. Ethanol concentrations and productivities ranged between 10.8–12.2 g $l^{-1}$ and 0.119–0.286 g $l^{-1}$ $h^{-1}$ respectively, over 50–121 h, in the detoxified hydrolysate media which contained 48.2 g $l^{-1}$ of total reducing sugar. Increase in inoculum concentration also improved the ethanol production yield.



## 1. Introduction

Bioethanol is an environment-friendly fuel as the carbon dioxide emitted after its combustion is absorbed by natural environment. It therefore does not disrupt the carbon dioxide balance in the atmosphere [1]. It is a liquid fuel that, when added to gasoline in amounts which will not cause modifications to it, can be used in existing vehicles [2]. In many countries, legislative regulations have been introduced to increase ethanol content of gasoline produced for use in the near future. Biomass; various agricultural products, side products and wastes, can be fermented to create ethanol after an initial pretreatment. Today, there exists well-established commercial processing technology for first generation bioethanol production from sucrose or starch containing feed stocks like sugar cane, corn and wheat. These raw materials currently cater to the current global demand for bioethanol [3]. However, as availability, cost and food safety issues impact raw material selection, the need to develop second generation ethanol production from lignocellulosic wastes becomes critical [4].

Lignocellulosic materials are mainly composed of cellulose, hemicellulose, lignin, ash and other extracted materials [5]. Cellulose and hemicellulose structures should be broken into fermentable sugars for ethanol production from lignocellulosic wastes [6]. Hydrolysis of the cellulose molecule results in glucose molecules while various sugars like xylose, arabinose, galactose, glucose and mannose are obtained through hemicellulose hydrolysis [7,8]. It has been found that the hydrolysis of hemicelluloses in woody structures leads mainly to mannose formation. However, xylose is the most abundant sugar following the hydrolysis of

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http://dx.doi.org/10.1016/j.fuel.2015.06.016
0016-2361/

hemicelluloses from agricultural wastes [9]. Ethanol production costs can be reduced by using hemicellulose in lignocellulosic masses efficiently [10].

Sugar beet pulp is a promising raw material for ethanol production containing, on average, a 28% hemicellulose content. In Turkey, approximately $4 \times 10^6$ tons/year sugar is produced where 90% of this production is sourced from sugar beet while 10% is from starch [11]. During this process, nearly 193.5 kg of wet sugar beet pulp is obtained from 1 ton of sugar beet [12]. As the considerable majority of bioethanol production is performed from sugar beet in Turkey, existing production plants could modified such that the side product of sugar beet pulp is utilized for ethanol production.

For hemicellulose conversion, dilute sulfuric acid hydrolysis is known to be an efficient and industrially-applicable method. The hydrolysate medium ingredients obtained from acidic hydrolysis are xylose (the main component), arabinose, glucose, galactose and mannose sugars and the inhibitory by-products include furan derivatives (furfural and 5-hydroxymethyl furfural), phenolic compounds and weak acids [13]. The efficiency of ethanol fermentation can be enhanced by detoxification of the hydrolysate and by using microorganisms with high tolerance toward inhibitor compounds [14]. *Pichia stipitis* is one of the limited number of naturally-occurring microorganisms that have the ability to ferment all of the glucose, xylose, mannose, galactose and cellobiose sugars, with high ethanol productivity [15]. Its thick cell wall and high resistance to contamination makes it suitable for industrial usage [16].

The main aim of this work was to test known and new detoxification methods for the removal of inhibitory compounds from sugar beet pulp hydrolysate obtained by dilute acid treatment. In this context, the effects of the detoxification methods on kinetic parameters related to cell growth rate, substrate consumption rate and the ethanol production capability of *P. stipitis* yeast in hydrosylate containing growth media were investigated. The results were compared to those obtained in the synthetic growth media of varying substrate concentrations.

## 2. Materials and methods

### 2.1. Preparation of hemicellulose hydrolysate

Sugar beet pulp, obtained from Ankara Sugar Factory in Turkey, was washed, dried in an oven at 100 °C for 48 h and milled. Particles within 0.707–1.000 mm size interval were hydrolyzed in 500 ml of 0.3 M sulfuric acid solution for 6 h, at 10:1 liquid/solid ratio in an agitated isothermal pyrex reactor at 110 °C. Optimum temperature and acid concentration values were determined after kinetic analysis of the reactions. In this context, a model proposing the decomposition of xylans to xylose and xylose to furfural was used to describe the reaction kinetics [17]. The filtered hydrolysate was used for detoxification processes after carrying out analyses to determine total reducing sugars, total furan compounds (furfural and 5-hydroxymethyl furfural), total phenolic compounds and acetic acid. Sugars formed by the hydrolysis of lignocellulosic beet pulp were measured as reducing sugars which may primarily contain xylose from the hemicellulose.

### 2.2. Detoxification of hydrolysate

The various treatments applied to the hydrolysates in the removal of inhibitors formed during hydrolysis are presented in Table 1.

Firstly, similar to the method which is described by Arslan [18]; hydrolysate was treated with 20 g $l^{-1}$ commercial activated charcoal (Merck: 0.15 mm particle size, 1456 $m^2$ $g^{-1}$ surface area and pore sizes of 1.07 nm and 3.70 nm) at 30 °C and 100 rpm shaking rate for 24 h. The filtered sample was overlimed by treating with CaO (Merck) until the medium pH reached 9–10 and then the pH was adjusted to 5.5–6 using concentrated $H_2SO_4$.

**Table 1**
Detoxification methods applied to the sugar beet pulp hydrolysate.

| No. | Detoxification method |
|---|---|
| 1 | Treatment with commercial activated charcoal + overliming[a] |
| 2 | Treatment with activated charcoal obtained from sugar beet pulp + overliming[b] |
| 3 | Treatment with fly ash[c] |

Secondly, the inhibitor compounds were adsorbed with activated charcoal which was obtained from sugar beet pulp as described by Sezer [19] with the pyrolysis of the sugar beet pulp particles smaller than 0.500 mm particle size (and with a 262 $m^2$ $g^{-1}$ surface area with pore sizes of 1.10 nm, 2.88 nm, 3.79 nm and 1.46 nm) in an $N_2$ atmosphere ash oven set at 750 °C. After the treatment of hydrolysate with 20 g $l^{-1}$ adsorbent for 24 h at 30 °C and 100 rpm, the overliming method was applied to the filtered sample.

Thirdly, the hydrolysate was treated with fly ash obtained from Afşin–Elbistan Thermal Power Plant (0.15 mm particle size, 1.55 $m^2$ $g^{-1}$ surface area) at the same experimental conditions with the other treatments. Following this the pH value of the medium recorded as 10–10.5 was reduced to 5.5–6 using concentrated $H_2SO_4$.

### 2.3. Microorganism and growth media

The *P. stipitis* NRRL Y-7124 strain which was used in this study was obtained from the United States Department of Agriculture. The yeast was grown at 30 °C in a synthetic growth medium containing 10 g $l^{-1}$ xylose, 3 g $l^{-1}$ yeast extract, 3 g $l^{-1}$ malt extract and 5 g $l^{-1}$ pepton at pH 4.5 [20,21]. After 72 h, the culture was transferred to 250 flasks containing 100 ml of experimental growth media composed of detoxified sugar beet pulp hydrolysates at varied quantities and/or other growth media including 10 g $l^{-1}$ synthetic D-xylose solution and other necessary nutrients as remarked by Nigam [4]. For sterilization, xylose and other nutrients were autoclaved separately. Hydrolysate was sterilized by filtering method in order to avoid sugar loss in it due to caramelization.

### 2.4. Adaptation of the yeast to the sugar beet pulp hydrolysate

The first inoculation was done to the medium with 20% hydrolysate by volume. When this culture reached its logarithmic growth phase, it was transferred to the medium with 40% hydrolysate by volume. This procedure was repeated for media with 60%, 80% and 100% hydrolysate by volume, respectively.

### 2.5. Fermentation

In order to determine kinetic parameters, fermentation studies were carried out with a 5% (v/v) inoculum concentration in the 100 ml of synthetic growth media that contained initial D-xylose concentrations between 10 and 75 g $l^{-1}$ in 250 ml-erlenmeyers. Following this, various growth media incorporating hydrolysate solutions (detoxified using methods 1, 2 and 3 (Table 1)) as sugar sources for cells adapted to hydrolysate medium were prepared. Cell growth, substrate consumption and ethanol production rates were investigated both in the media of 10 g $l^{-1}$ synthetic D-xylose with hydrolysate and in the media with hydrolysate as the only source of sugar. All the experiments were performed at 30 °C and at 100 rpm in a shaking incubator.



Please cite this article in press as: Gunan-Yucel H, Aksu Z. Ethanol fermentation characteristics of *Pichia stipitis* yeast from sugar beet pulp hydrolysate: Use of new detoxification methods. Fuel (2015), http://dx.doi.org/10.1016/j.fuel.2015.06.016